

Sorting by pools

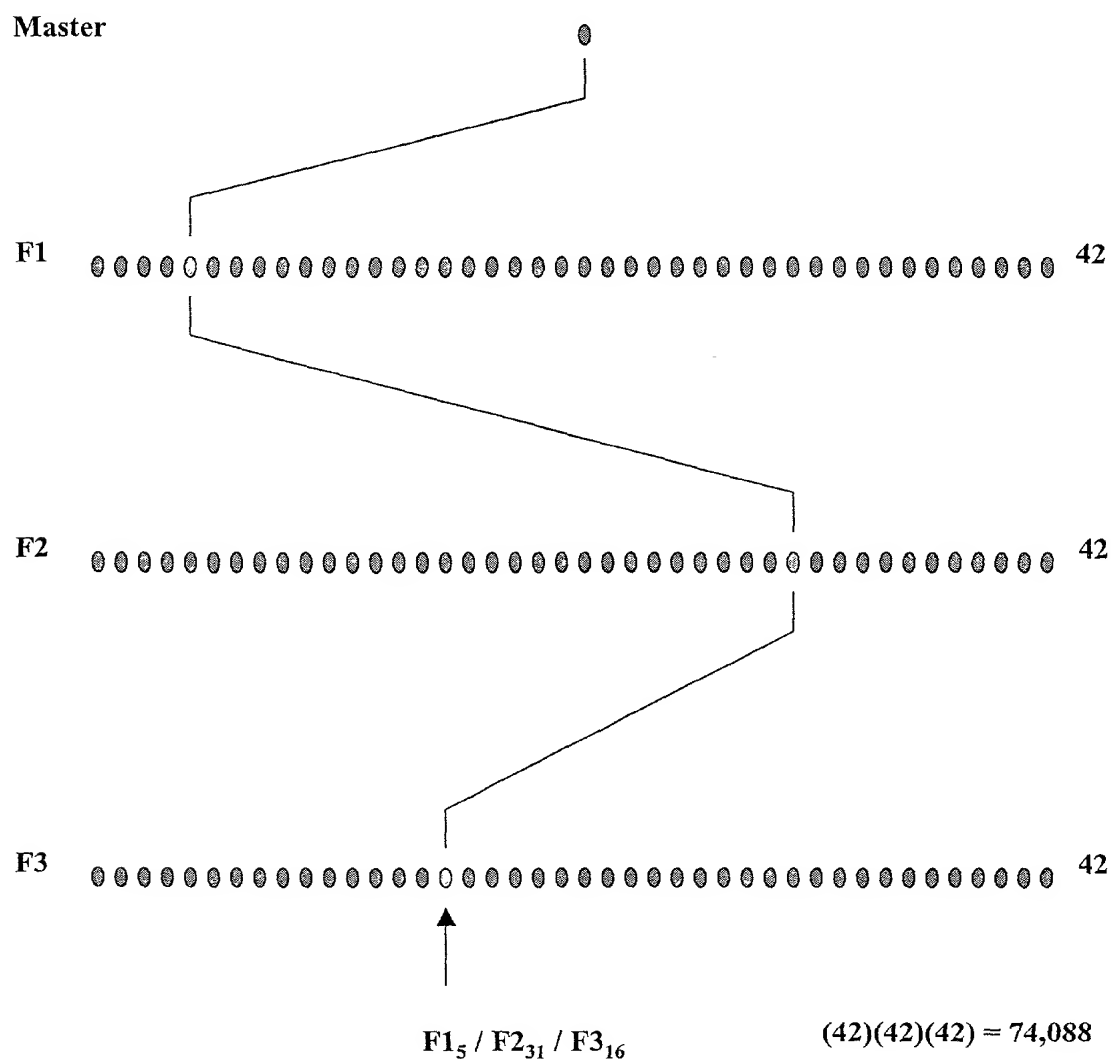


FIGURE 1

Sorting by pools: Decreasing pool diversities

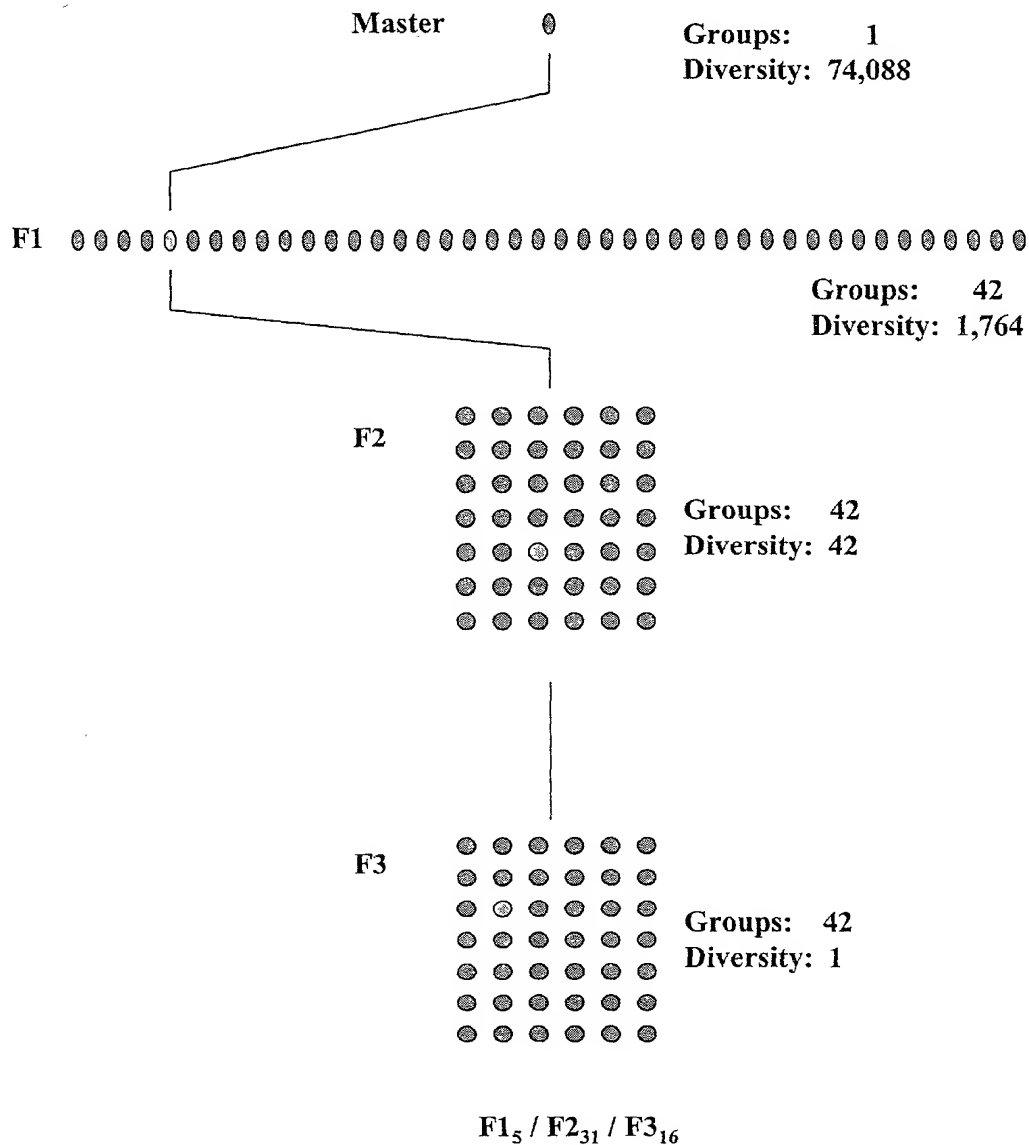


FIGURE 2

Sorting by pools: Screening large diversity libraries

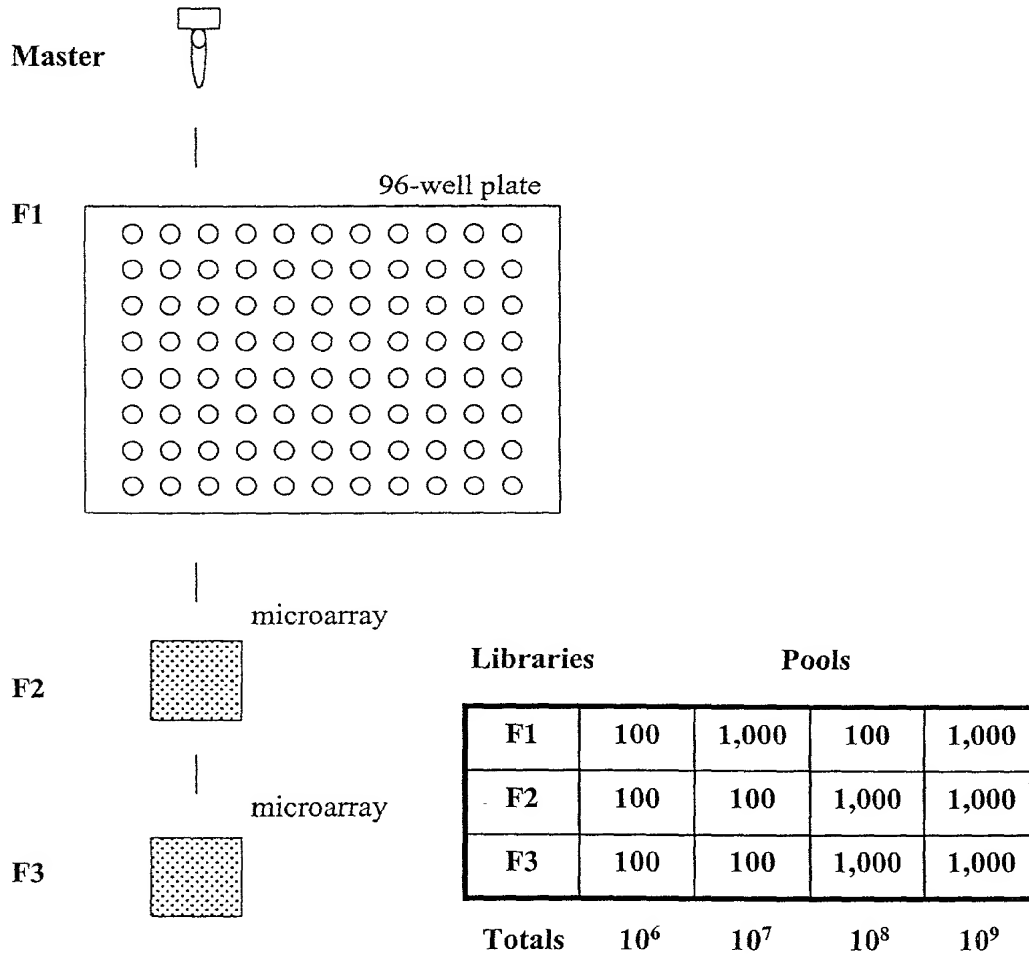


FIGURE 3

Searching a mutation library

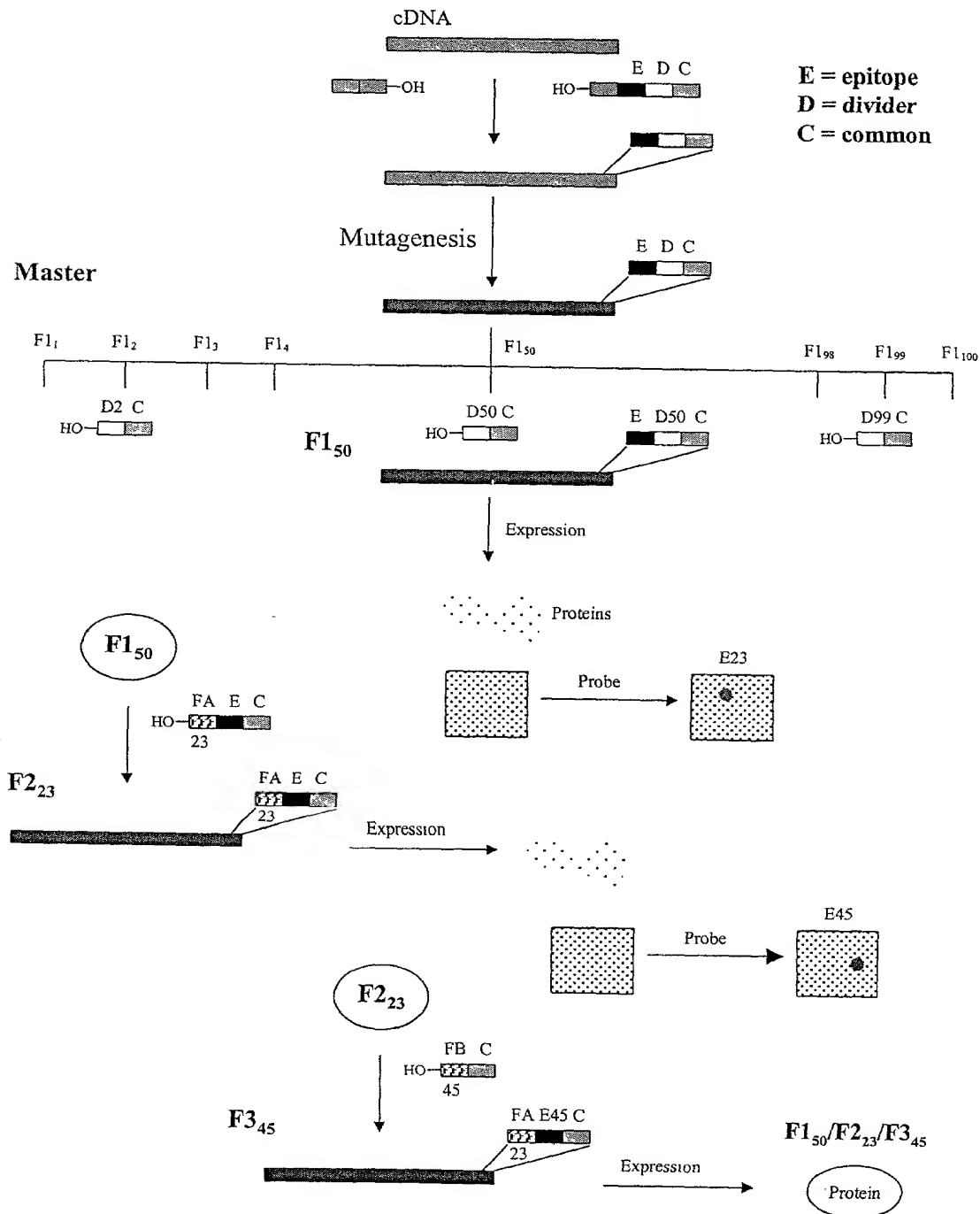
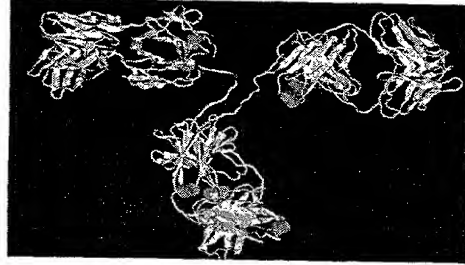
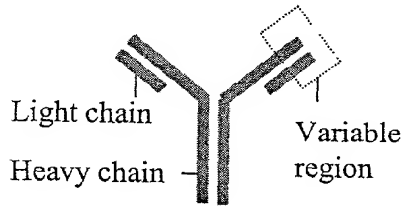


FIGURE 4

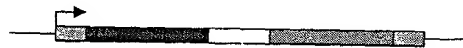
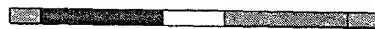
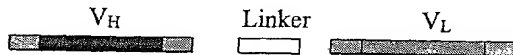
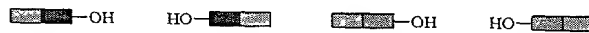
Applicant: Ault-Riche *et al.*
DKT. No. 25885-1751
Priority claimed to 60/219,183
For: COLLECTIONS OF BINDING PROTEINS AND
TAGS AND USES THEREOF FOR NESTED SORTING
AND HIGH THROUGHPUT SCREENING

Making a recombinant antibody library

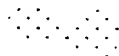
Basic antibody structure



Spleen cells or PBLs



Expression



Antibodies

FIGURE 5

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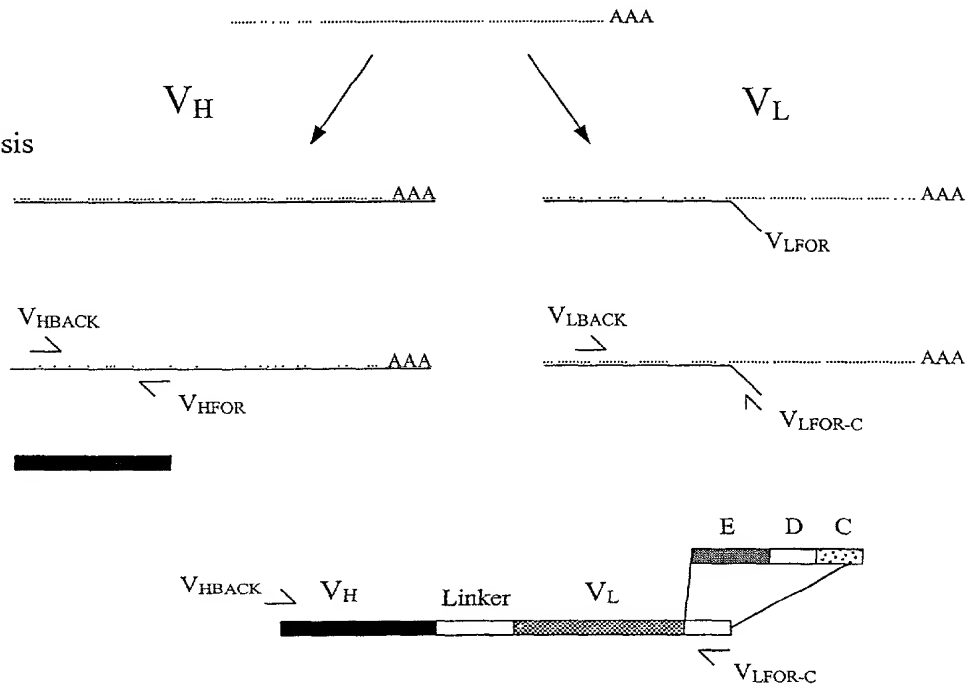
Creating the master antibody library: Primer incorporation

1. mRNA purification from spleen or PBLs

2. cDNA synthesis

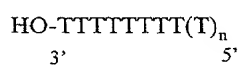
3. amplification

4. assembly

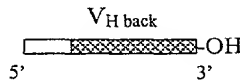


V_H Primers

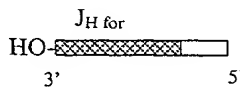
Oligo dT



V_HBACK

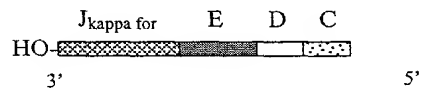


V_HFOR

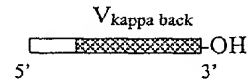


V_L Primers

V_LFOR



V_LBACK



V_LFOR-C

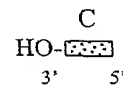


FIGURE 6

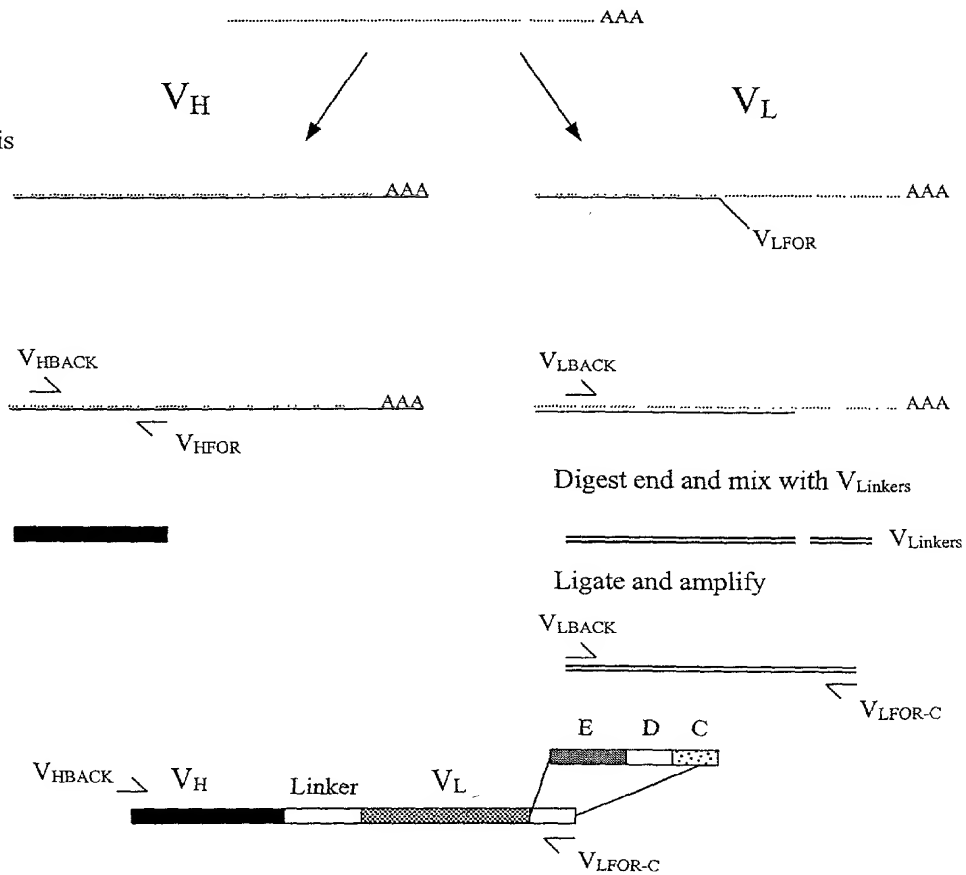
Creating the master antibody library: Linker addition

1. mRNA purification from spleen or PBLs

2. cDNA synthesis

3. amplification

4. assembly



V_H Primers

Oligo dT HO-TTTTTTTT(T)_n 3' 5'

V_HBACK 5' V_H back 3'-OH

V_HFOR HO- J_H for 3' 5'

V_L Primers

V_LFOR HO- J_{kappa} for 3' 5'

V_LBACK 5' V_{kappa} back 3'-OH

$V_{Linkers}$ HO- J_{kappa} for E D C 3' 5'

V_LFOR -C HO-C 3' 5'

FIGURE 7

Searching a recombinant antibody library

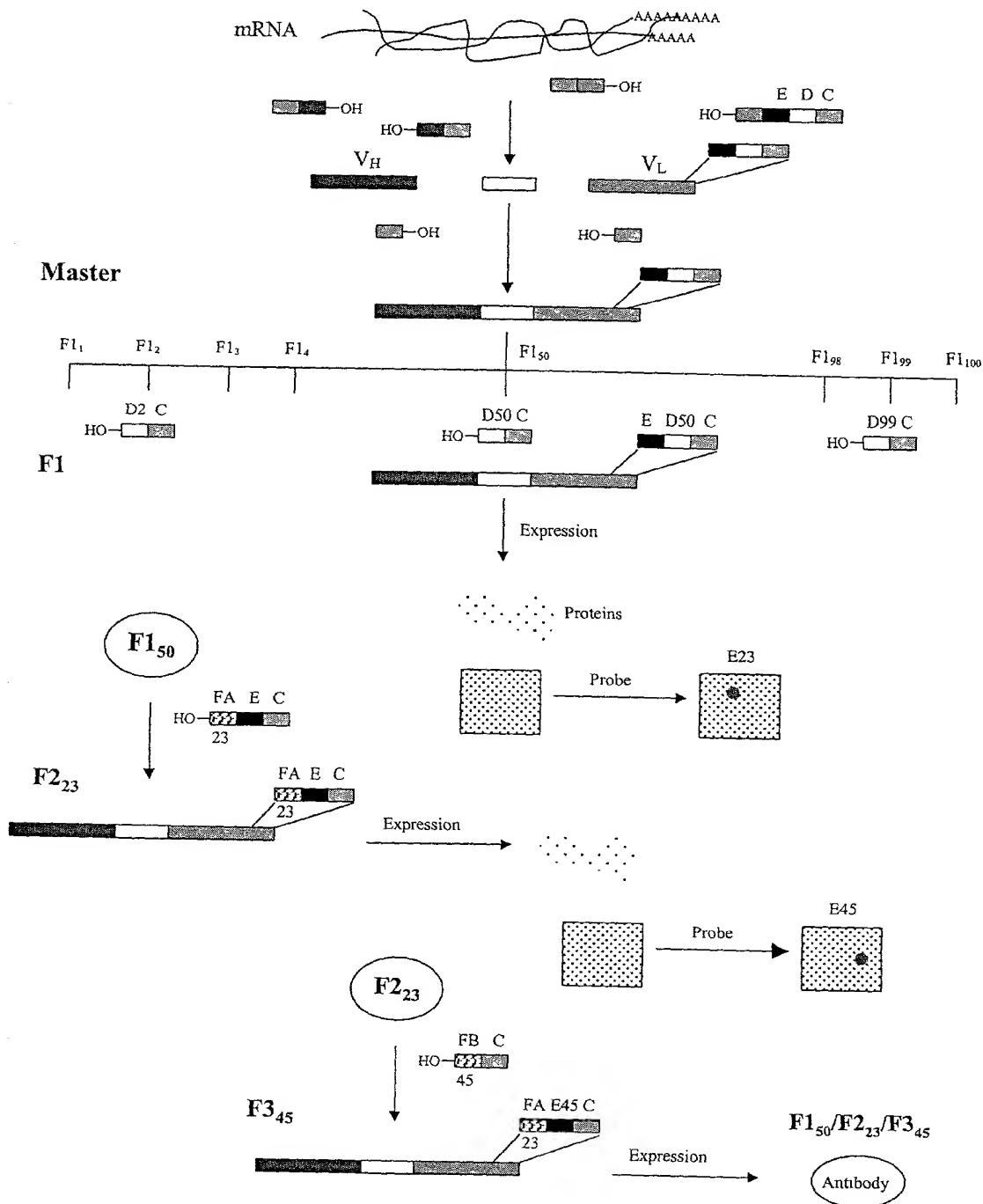
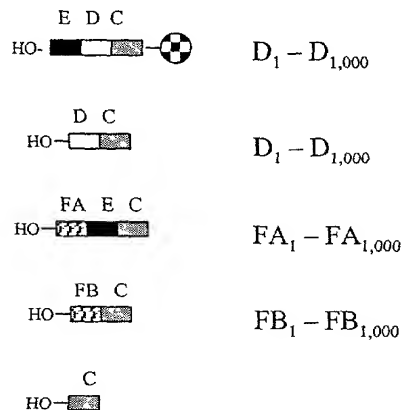


FIGURE 8

Physical elements to include in the kits and combinations

- *Anti-tag Arrays*[™]

- Primer sets

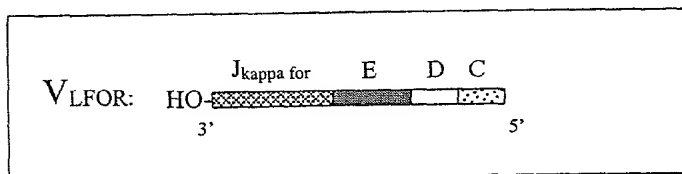


- Readers

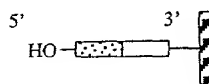
- Software

FIGURE 9

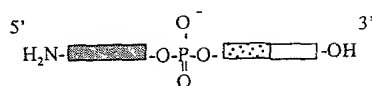
Making the V_{LFOR} primers: Solid phase synthesis



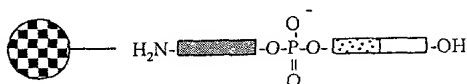
1. Synthesize oligo on solid support



2. Add aminolink prior to cleavage



3. Couple to tosyl activated magnetic beads



4. Extend by hybridizing with DNA patch and ligating

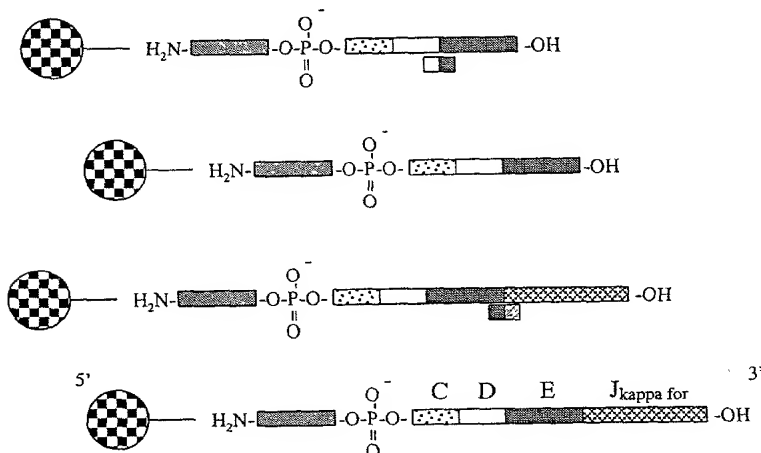
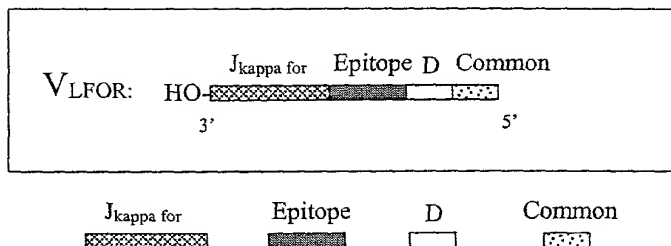


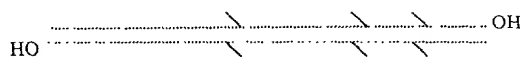
FIGURE 10

Making the V_LFOR primers: Overlapping hybridization

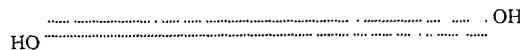


- Synthesize 4,028 different oligos:
 (26 for J_{kappa} for; 2,000 for Epitope; 2,000 for D; 2 for Common)

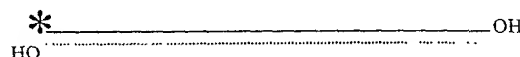
2. Assemble oligos for + and - strands of the different regions



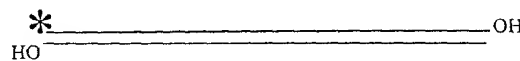
3. Ligase the assembled oligos



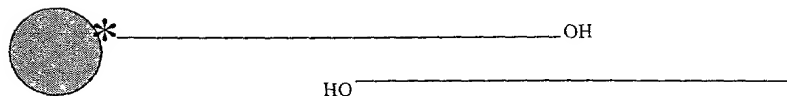
4. 1st strand synthesis with biotinylated primer



- 2nd strand synthesis with non-biotinylated primer



6. Bind to avidin coated magnetic beads and then denature



7. Purify non-biotinylated ssDNA

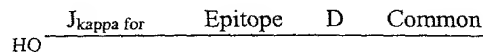


FIGURE 11

Building the collection of antibody/tag pairs: Hybridoma screening

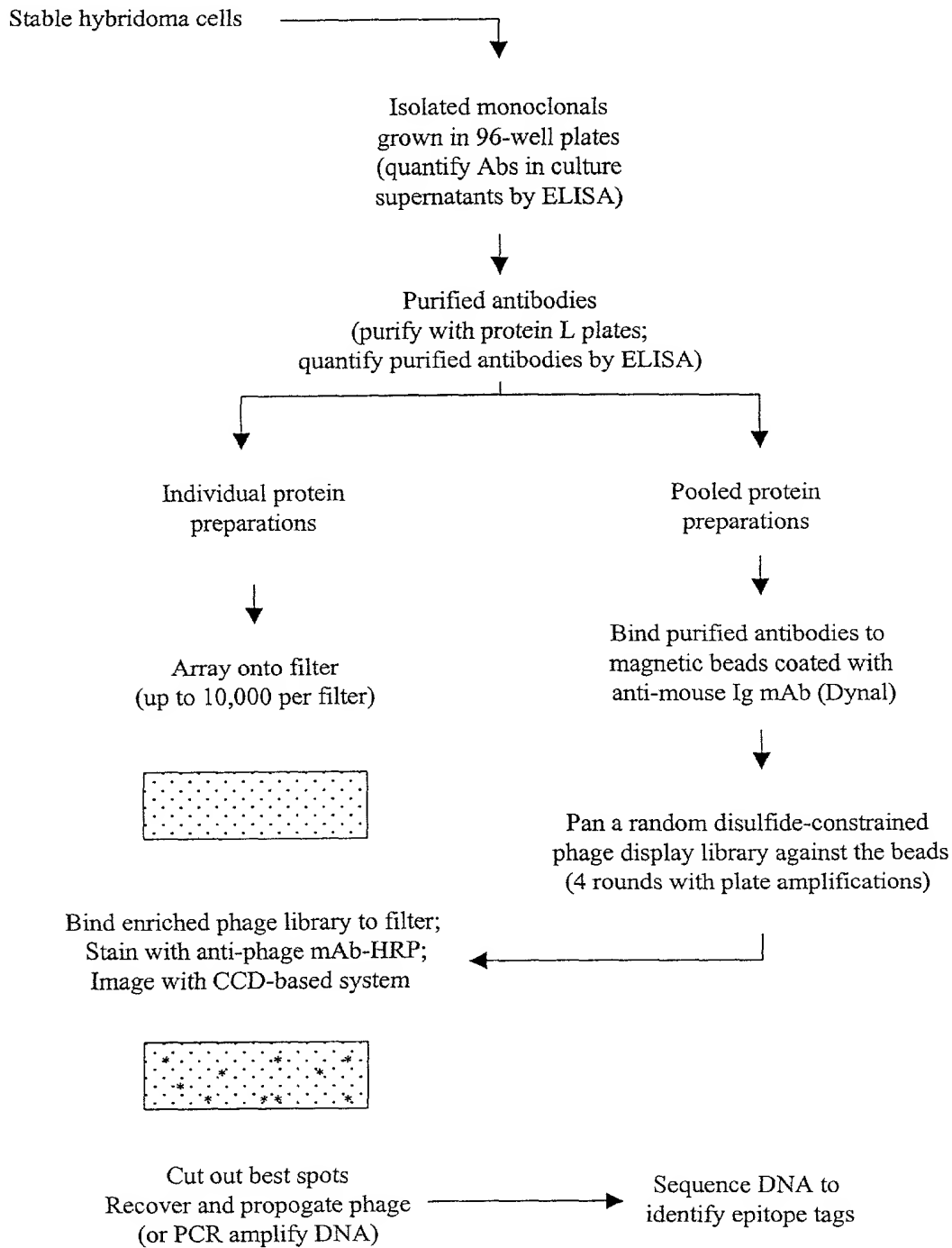


FIGURE 12

FIGURE 13A

TABLE 3 Primers for PCR Amplification of Human Antibody Variable Regions (V genes)

1. V gene primary PCR

A. Human VH back primers (sense)

HuVH1aBACK	5'-CAG GTG CAG CTG GTG CAG TCT GG-3'
HuVH2aBACK	5'-CAG GTC AAC TTA AGG GAG TCT GG-3'
HuVH3aBACK	5'-GAG GTG CAG CTG GTG GAG TCT GG-3'
HuVH4aBACK	5'-CAG GTG CAG CTG CAG GAG TCG GG-3'
HuVH5aBACK	5'-GAG GTG CAG CTG TTG CAG TCT GC-3'
HuVH6aBACK	5'-CAG GTA CAG CTG CAG CAG TCA GG-3'

B. Human JH forward primers (anti-sense)

HuJH1-2FOR	5'-TGA GGA GAC GGT GAC CAG GGT CCC-3'
HuJH3FOR	5'-TGA AGA GAC GGT GAC CAT TGT CCC-3'
HuJH4-5FOR	5'-TGA GGA GAC GGT GAC CAG GGT TCC-3'
HuJH6FOR	5'-TGA GGA GAC GGT GAC CGT GGT CCC-3'

C. Human V kappa back primers (sense)

HuVk1aBACK	5'-GAC ATC CAG ATG ACC CAG TCT CC-3'
HuVk2aBACK	5'-GAT GTT GTG ATG ACT CAG TCT CC-3'
HuVk3aBACK	5'-GAA ATT GTG TTG ACG CAG TCT CC-3'
HuVk4aBACK	5'-GAC ATC GTG ATG ACC CAG TCT CC-3'
HuVk5aBACK	5'-GAA ACG ACA CTC ACG CAG TCT CC-3'
HuVk6aBACK	5'-GAA ATT GTG CTG ACT CAG TCT CC-3'

C. Human V lambda back primers (sense)

HuVL1BACK	5'-CAG TCT GTG TTG ACG CAG CCG CC-3'
HuVL2BACK	5'-CAG TCT GCC CTG ACT CAG CCT GC-3'
HuVL3aBACK	5'-TCC TAT GTG CTG ACT CAG CCA CC-3'
HuVL3bBACK	5'-TCT TCT GAG CTG ACT CAG GAC CC-3'
HuVL4BACK	5'-CAC GTT ATA CTG ACT CAA CCG CC-3'
HuVL5BACK	5'-CAG GCT GTG CTC ACT CAG CCG TC-3'
HuVL6BACK	5'-AAT TTT ATG CTG ACT CAG CCC CA-3'

D. Human J kappa forward primers (anti-sense)

HuJk1FOR	5'-ACG TTT GAT TTC CAC CTT GGT CCC-3'
HuJk2FOR	5'-ACG TTT GAT CTC CAG CTT GGT CCC-3'
HuJk3FOR	5'-ACG TTT GAT ATC CAC TTT GGT CCC-3'
HuJk4FOR	5'-ACG TTT GAT CTC CAC CTT GGT CCC-3'
HuJk5FOR	5'-ACG TTT AAT CTC CAG TCG TGT CCC-3'

D. Human J lambda forward primers (anti-sense)

HuJλ1FOR	5'-ACC TAG GAC GGT GAC CTT GGT CCC-3'
HuJλ2-3FOR	5'-ACC TAG GAC GGT CAG CTT GGT CCC-3'
HuJλ4-5FOR	5'-ACC TAA AAC GGT GAG CTG GGT CCC-3'

continues

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FIGURE 13B

TABLE 3 Continued

2. Linker fragment PCR

F. Reverse JH for scFv linker (sense)

FR4 heavy ————— | — linker
 RHuJH1-2 5'-GC ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH3 5'-GG ACA ATG GTC ACC GTC TCT TCA GGT GG-3'
 RHuJH4-5 5'-GA ACC CTG GTC ACC GTC TCC TCA GGT GG-3'
 RHuJH6 5'-GG ACC ACG GTC ACC GTC TCC TCA GGT GG-3'

F. Reverse Vk for scFv linker (anti-sense)

FR1 light ————— | — linker—
 RHuVk1aBACKFv 5'-GG AGA CTG GGT CAT CTG GAT GTC CGA TCC GCC-3'
 RHuVk2aBACKFv 5'-GG AGA CTG AGT CAT CAC AAC ATC CGA TCC GCC-3'
 RHuVk3aBACKFv 5'-GG AGA CTG CGT CAA CAC AAT TTC CGA TCC GCC-3'
 RHuVk4aBACKFv 5'-GG AGA CTG GGT CAT CAC GAT GTC CGA TCC GCC-3'
 RHuVk5aBACKFv 5'-GG AGA CTG CGT GAG TGT CGT TTC CGA TCC GCC-3'
 RHuVk6aBACKFv 5'-GG AGA CTG AGT CAG CAC AAT TTC CGA TCC GCC-3'

F. Reverse VL for scFv linker (anti-sense)

FR1 light ————— | — linker—
 RHuVLBACK1Fv 5'-GG CGG CTG CGT CAA CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK2Fv 5'-GC AGG CTG AGT CAG AGC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK3aFv 5'-GG TGG CTG AGT CAG CAC ATA GGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK3bFv 5'-GG GTC CTG AGT CAG CTC AGA AGA CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK4Fv 5'-GG CGG TTG AGT CAG TAT AAC GTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK5Fv 5'-GA CGG CTG AGT CAG CAC AGA CTG CGA TCC GCC ACC GCC AGA G-3'
 RHuVLBACK6Fv 5'-TG GGG CTG AGT CAG CAT AAA ATT CGA TCC GCC ACC GCC AGA G-3'

3. Pull-through primers for introduction of restriction sites*

G. Human VH back (Sfi) primers (sense)

FR1 heavy —————
 HuVH1aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG GTG CAG TCT GG-3'
 HuVH2aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTC AAC TTA AGG GAG TCT GG-3'
 HuVH3aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC GAG GTG CAG CTG GTG GAG TCT GG-3'
 HuVH4aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG CAG GAG TCG GG-3'
 HuVH5aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTG CAG CTG TTG CAG TCT GC-3'
 HuVH6aBACKSfi 5'-GTC CTC GCA ACT GCG GCC CAG CCG GCC ATG GCC CAG GTA CAG CTG CAG CAG TCA GG-3'

H. Human J kappa forward (Not) primers (anti-sense)

FR4 light —————
 HuJk1FORNot 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACG TTT GAT TTC CAC CTT GGT CCC-3'
 HuJk2FORNot 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACG TTT GAT CTC CAG CTT GGT CCC-3'

H. Human J kappa forward (Not) primers (anti-sense) (Continued)

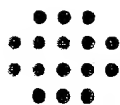
FR4 light —————
 HuJk3FORNot 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACG TTT GAT ATC CAC TTT GGT CCC-3'
 HuJk4FORNot 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACG TTT GAT CTC CAC CTT GGT CCC-3'
 HuJk5FORNot 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACG TTT AAT CTC CAG TCG TGT CCC-3'

H. Human J lambda forward (Not) primers (anti-sense)

FR4 light —————
 HuJl1FORNOT 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACC TAG GAC GGT GAC CTT GGT CCC-3'
 HuJl2-3FORNOT 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACC TAG GAC GGT CAG CTT GGT CCC-3'
 HuJl4-5FORNOT 5'-GAG TCA TTC TCG ACT TGC GCC CGC ACC TAA AAC GGT GAG CTG GGT CCC-3'

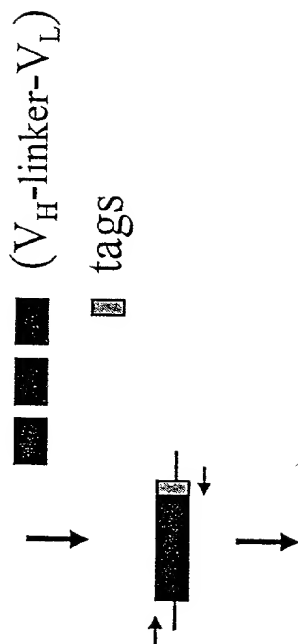
*Recognition site for restriction enzyme is underlined.

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step I

Tag and assemble immunoglobulin genes



Create 1,000 sub-libraries by separate PCR amplification reactions using tag-specific PCR primers



1,000 sub-libraries

FIGURE 14A

step II

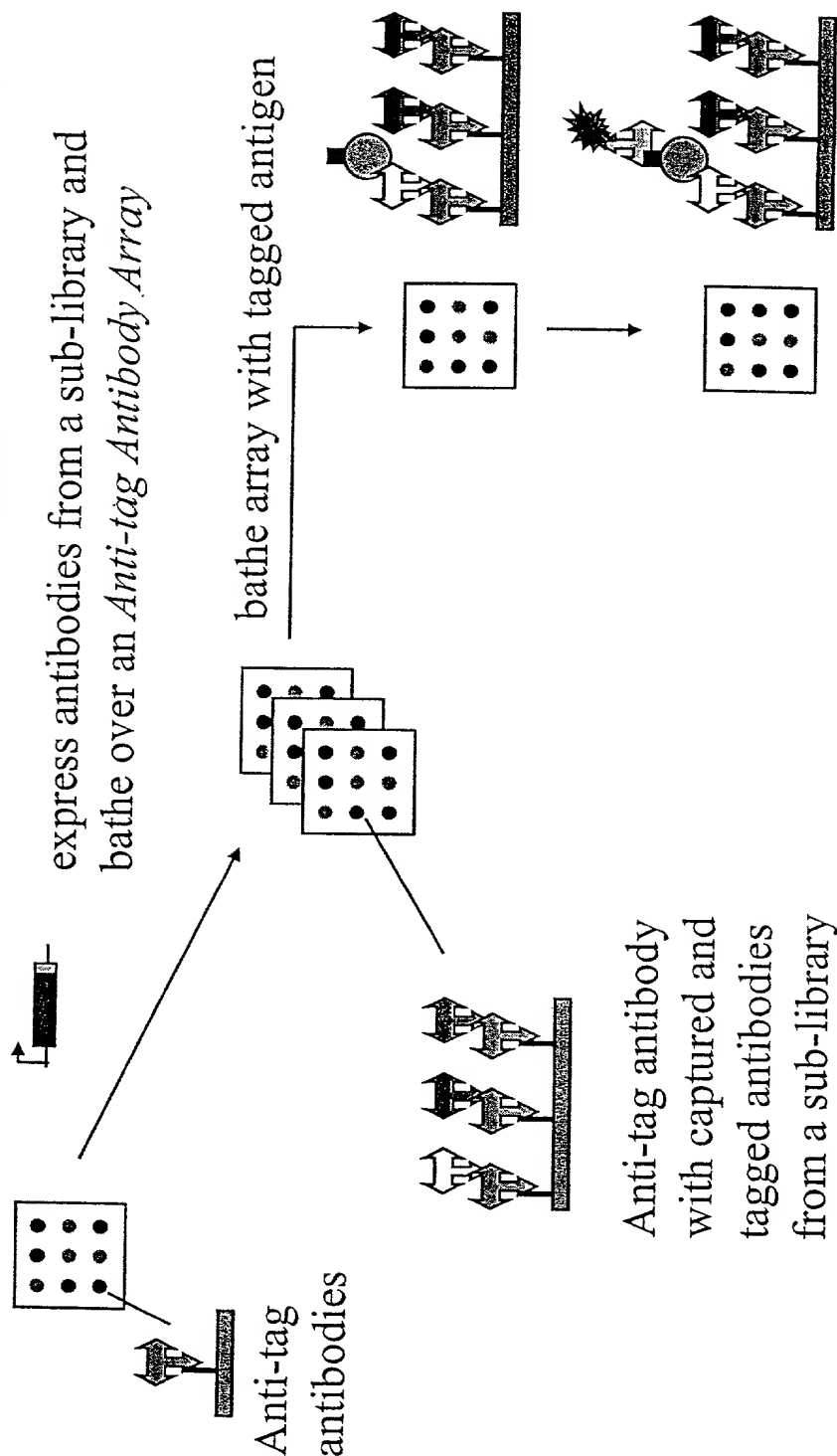
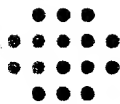


FIGURE 14B



step III

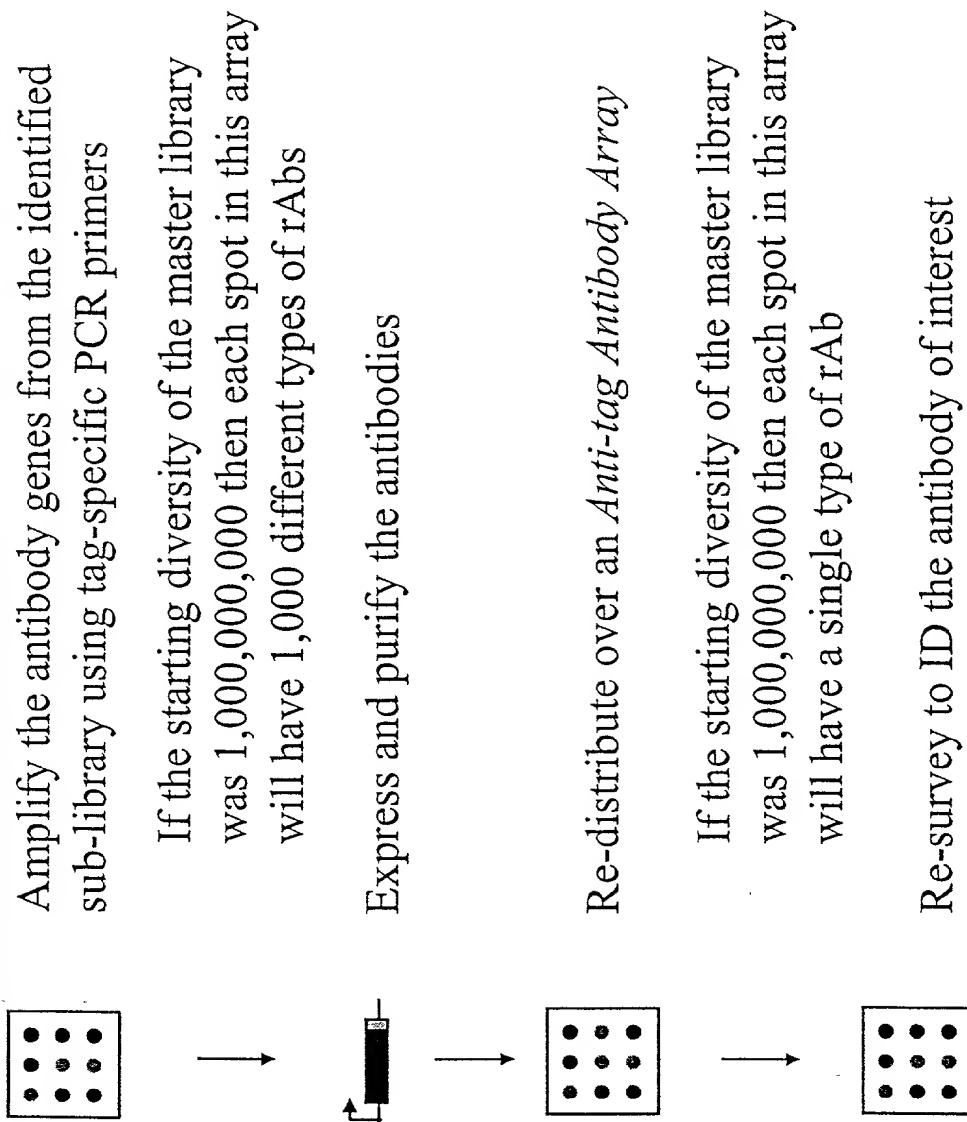
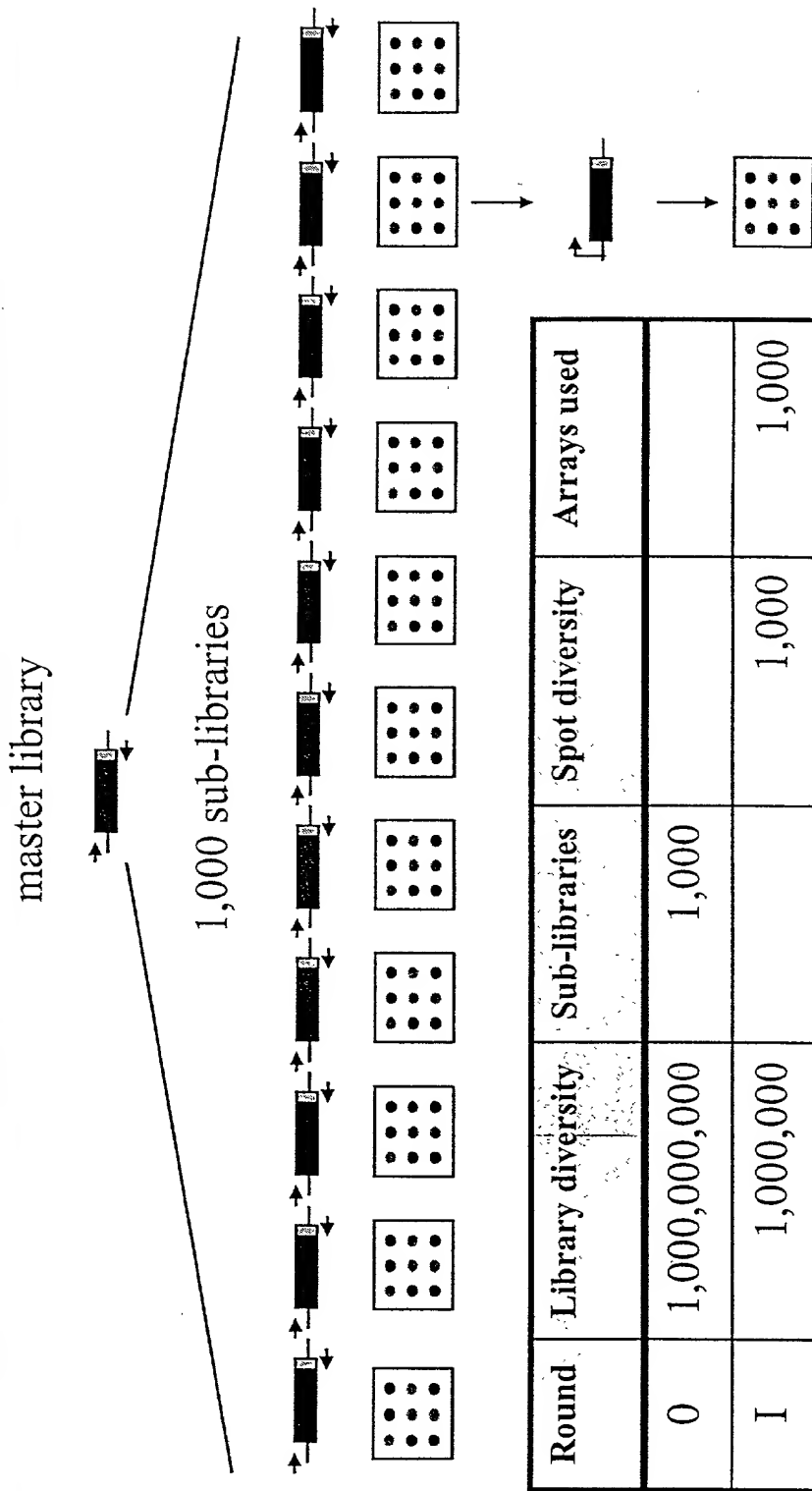


FIGURE 14C

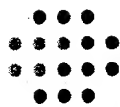
108120' 02101660

summary



Round	Library diversity	Sub-libraries	Spot diversity	Arrays used
0	1,000,000,000	1,000		
I	1,000,000		1,000	1,000
II	1,000		1	1

FIGURE 14D



- Modification searches

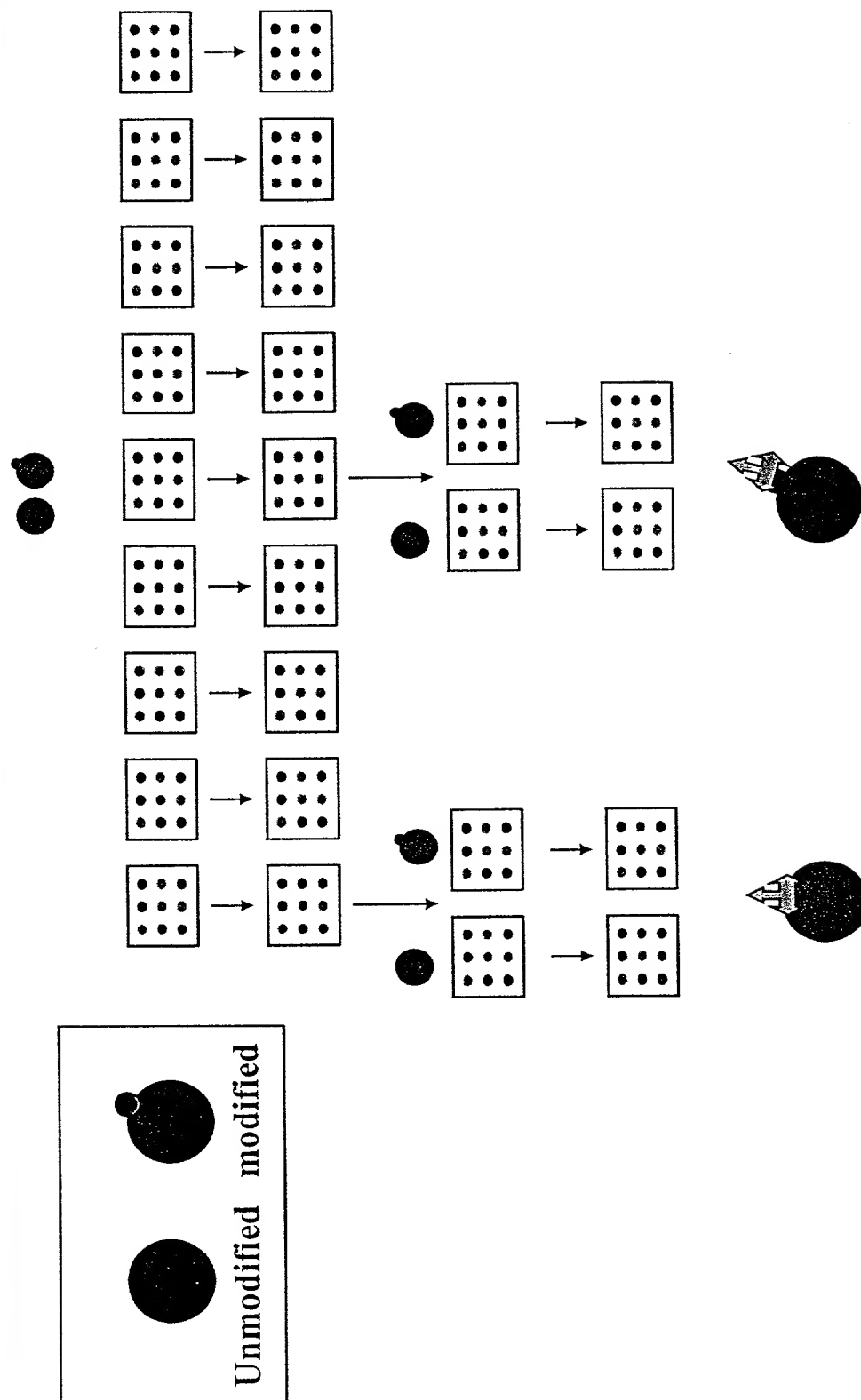
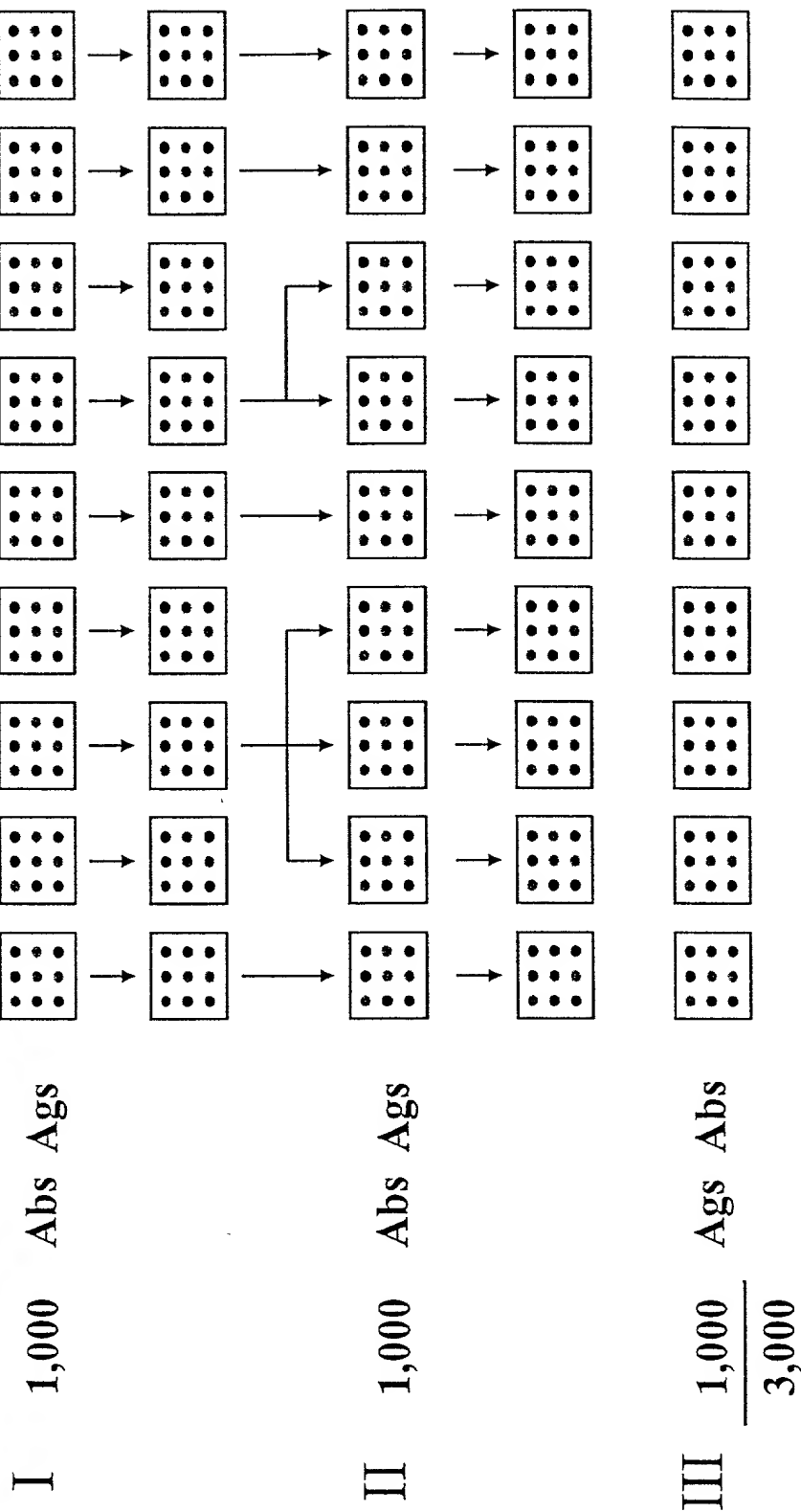


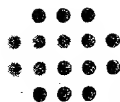
FIGURE 15

Round Arrays Bait Probe



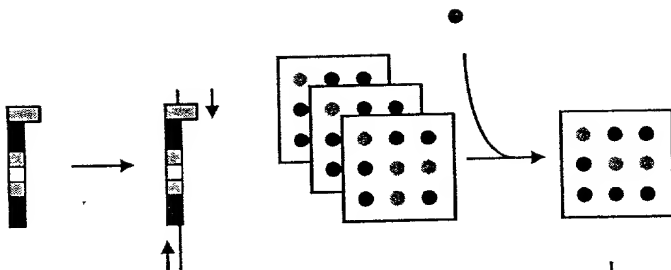
3 Arrays per Ag

FIGURE 16

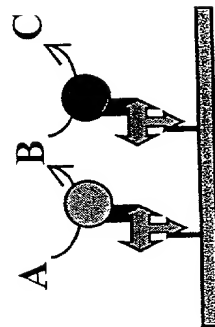


Enzyme engineering

Natural gene(s) → Error-prone PCR or Gene Shuffling → Mutated genes

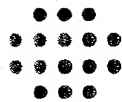


- tag the gene to be mutated
- mutate genes and create sub-libraries
- distribute mutants over arrays
- probe the arrays with labeled substrates



Spots can contain mixtures of enzymes for detection or pathway engineering

FIGURE 17



Protein interaction mapping

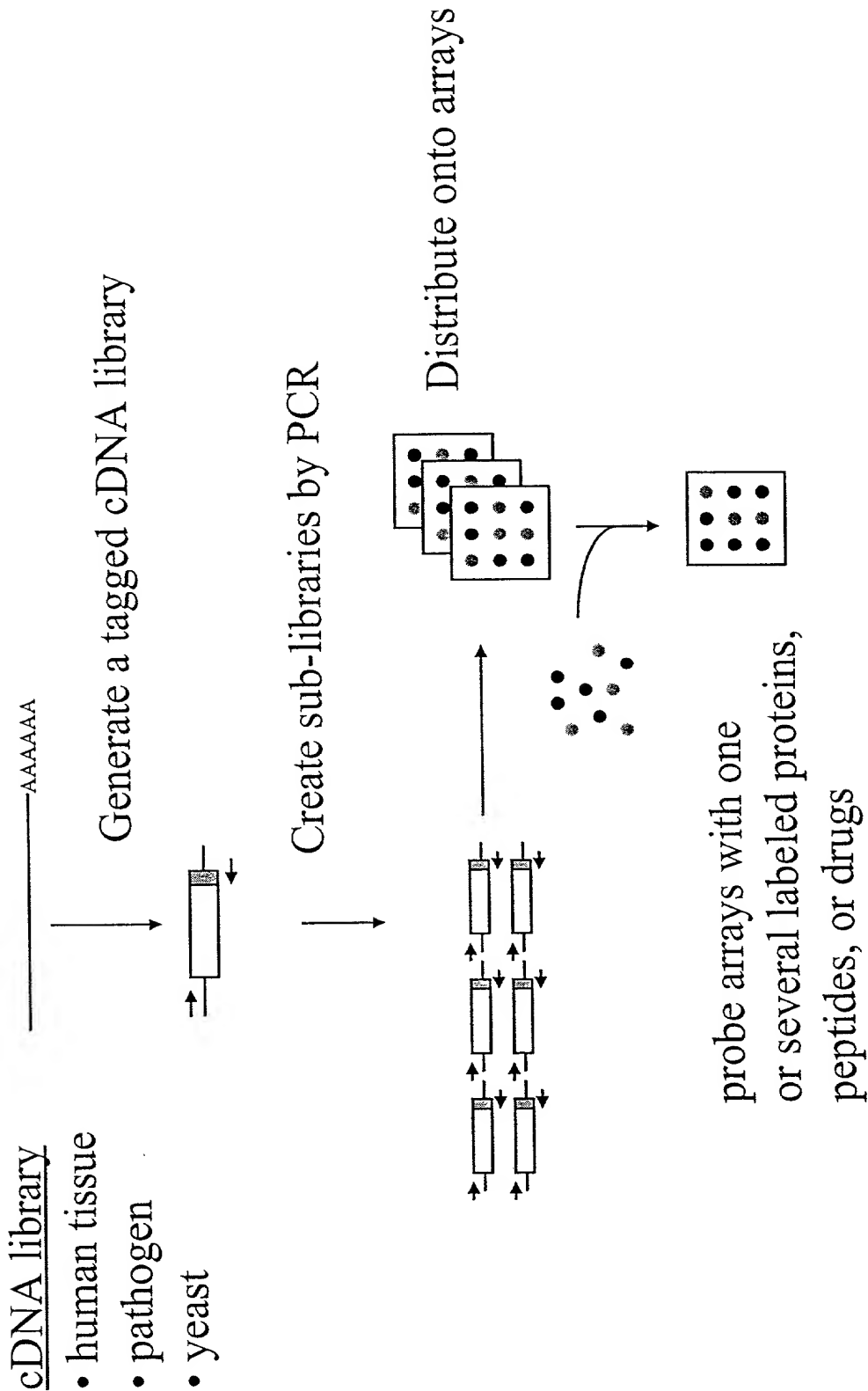
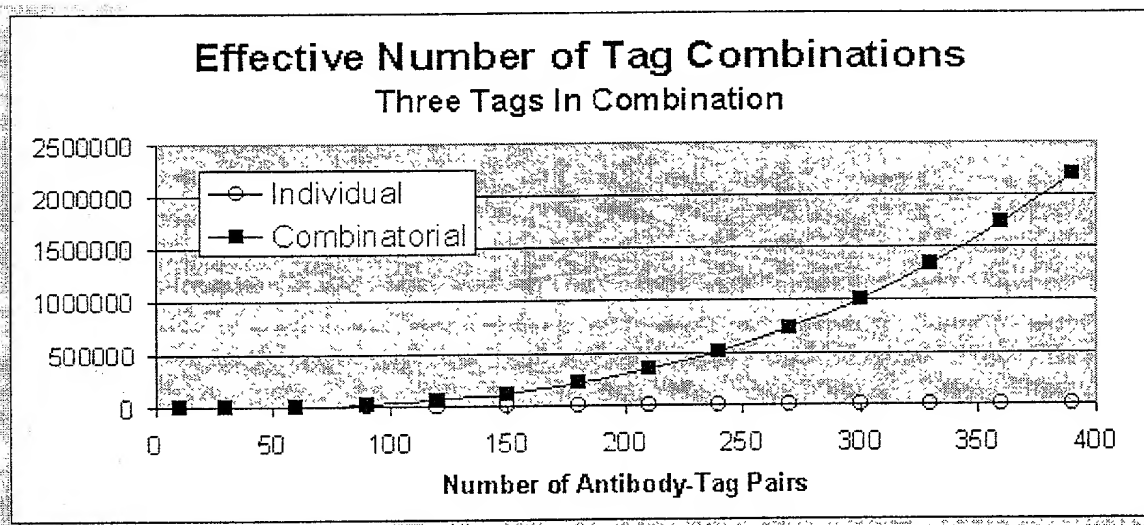


FIGURE 18

FIGURE 19



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T08T20-02T01560